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FINAL REPORT

TO: U.S. Office of Naval Research Molecular Biology Program

CONTRACT: The Role of Microorganisms in Marine Corrosion

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Our research under this contract was designed to investigate the role of microorganisms in hydrogen embrittlement. Specific objectives included the quantification of the amounts of microbially produced hydrogen absorbed by sensitive metals; the effect of competition from microbially produced hydrogen between metal surfaces and hydrogen consuming bacteria; and the effect of microbial metabolites on hydrogen absorption by metals.

The first stage of this research involved the adaptation of a conventional electrochemical technique in order to provide a quantitative assay of hydrogen production in anaerobic zones between biofilms and metal surfaces. In this first stage of the work, we calculated hydrogen production on a per-cell basis. In all cases we used palladium foil. The advantage of this metal is that hydrogen permeation is not complicated by secondary phases within the metal, and absorption efficiency is very high. Maximum production of hydrogen was calculated at 6.5 x 10 -11 moles per cell.

We carried out direct counts of bacteria on the palladium surface. Our data showed that the bacterial films were very thin. However, those organisms that were attached to the metal were metabolically extremely active, fermenting glucose at a rate at least ten-fold that of freely suspended cells. The high activity is probably caused by absorption of atomic hydrogen by the palladium foil, removing an inhibitory metabolic product.

We tested the permeation and growth parameters of a number of different anaerobic bacteria growing in biofilms on the palladium surface. Species of Clostridium produced high concentrations of hydrogen. However, hydrogen production by Desulfovibrio sulfuricans was negligible, despite considerable numbers of organisms attached to the surface. The palladium surface allowed us to characterize hydrogen production rates in biofilms. The permeation characteristics of Clostridium reflect metabolic processes which differ from other anaerobic bacteria. For example, Clostridium acetobutylicum displayed a rapid decrease in hydrogen production in the biofilm, reflecting a movement to hydrogen utilization. In the case of Desulfovibrio the hydrogen appeared to be very tightly cycled in sulfate reduction so that no net hydrogen production occured. In addition, formation of a thick layer of ferrous sulfide on the palladium membrane also inhibited hydrogen permeation when the solution was subsequently saturated with hydrogen gas.

These data were used to assess the possibility of embrittlement of susceptible materials by microbially produced hydrogen. We calculated the input concentration of hydrogen to palladium from the permeation current density using Fick's first law. We found that the

effect of hydrogen concentration beneath our bacterial films was close to the concentration required to produce crack propagation in high strength steel. In addition, hydrogen gas pressure under individual bacterial cells may be much higher than the average pressure derived from the total membrane surface.

The large permeation currents measured through the use of palladium permited us to make in-depth analyses of the relationship between the activities of bacteria in biofilms and hydrogen absorption. The modified Devanathan cell, which we used in our studies, provided new insights into the role of bacteria in hydrogen embrittlement processes of alloys in contact with aqueous environments.

We set up two stress testing systems to study the surface characteristics and failure rates of steels exposed to cultures of hydrogen producing bacteria. The first system was designed to test stainless steel wire under variable loading conditions. In this system a weight was suspended vertically from one end of the wire which passed through a microbiological cell. Initial studies showed a considerable decrease in the open circuit potential of the metal on exposure to anaerobic cultures. The other system was designed to test steel rods that were stressed close to their maximum tensile strength. The rod was placed horizontally and a weight was suspended at a predetermined distance from the microbiological cell in order to create the desired stress at a specific point on the upper surface of the rod. Our studies using these systems yielded longitudinal cracks in the rods within two months. In all cases, the cracks were related to growth of the anaerobic bacteria. Sterile rods did not crack.

Electron microscopy revealed the growth of the bacteria along the surfaces where the cracks occurred and within the cracks. It appears that hydrogen producing bacteria were responsible for stress induced failure of these materials. Further research is required to determine if this microbially induced failure occurs under normal conditions where mixed communities of bacteria are present.

PUBLICATIONS

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